AMENDMENTS TO THE SPECIFICATION

Please make the following changes to the application as published (US 2002/0102686 A1):

[0001] This application is a continuation of U.S. patent application Ser. No. 09/128,354, filed Aug. 3, 1998, ~~pending~~ now U.S. Patent No. 6,337,200; which is a continuation-in-part of U.S. patent application Ser. No. 09/052,864, filed Mar. 31, 1998, now abandoned.

[0002] The aforelisted priority applications are hereby incorporated herein by reference in their entirety, as are the following: U.S. patent application Ser. Nos. 08/851,843; 08/854,050; 08/911,312; 08/912,951; 08/915,503; 08/974,549; and 08/974,584; and ~~International Applications PCT/US97/17618 and PCT/US97/17885, which designate the U.S. and were published on Oct. 1, 1998~~ International Patent Publications WO 98/14592 and WO 98/14593.

[0010] In a different aspect of the invention, an isolated or recombinant hTRT polypeptide that has a deletion of amino acid residues 192-450, 560-565, 637-660, 638-660, 748-766, 748-764, or 1055-1071, where the residue numbering is with reference to the hTRT polypeptide having the sequence provided in FIG. 1, is provided. In one embodiment, the hTRT protein fragment has at least 6 amino acid residues. In other embodiments, the hTRT protein fragment has at least 8, at least about 10, at least about 12, at least about 15, or at least about 20 contiguous amino acid residues of a naturally occurring hTRT polypeptide. In still other embodiments, the hTRT protein fragment has at least about 50 or at least about 100 amino acid residues. In a related aspect, the invention provides an isolated, recombinant, or substantially purified polynucleotide encoding this polypeptide, which in some embodiments includes a promoter sequence operably linked to the nucleotide sequence encoding the hTRT polypeptide.

[0016] Telomerase is a ribonucleoprotein complex (RNP) comprising an RNA component and a catalytic protein component. The catalytic protein component of human telomerase, hereinafter referred to as telomerase reverse transcriptase ("hTRT"), has been cloned, and protein, cDNA and genomic sequences determined. See, e.g., Nakamura et al., 1997, Science 277:955, and ~~copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178. The sequence of a full-length native hTRT has been deposited in GenBank (Accession No. AF015950), and plasmid and phage vectors having hTRT coding sequences have been deposited with the American Type Culture Collection, Rockville, Md. (accession numbers 209024, 209016, and 98505). The catalytic subunit protein of human telomerase has also been referred to as "hEST2" (Meyerson et al., 1997, Cell 90:785), "hTCS1" (Kilian et al., 1997, Hum. Mol. Genet. 6:2011), "TP2" (Harrington et al., 1997, Genes Dev. 11:3109), and "hTERT" (e.g., Greider, 1998, Curr. Biol 8:R178-R181). Human TRT is also described in the aforereferenced priority applications and U.S. patent application Ser. Nos. 08/846,017, 08/844,419, and 08/724,643. The RNA component of human telomerase (hTR) has also been characterized (see U.S. Pat.

No. 5,583,016). All of the aforementioned applications and publications are incorporated by reference herein in their entirety and for all purposes.

[0017] Human TRT is of extraordinary interest and value because, inter alia, telomerase activity in human cells and other mammalian cells correlates with cell proliferative capacity, cell immortality, and the development of a neoplastic phenotype. Thus, hTRT polypeptides, including the hTRT variants described herein, and polynucleotides encoding hTRT polypeptides, are used, inter alia, for conferring a telomerase activity (e.g., telomerase catalytic activity, infra) in a telomerase-negative cell such as a cell from a human, a mammal, a vertebrate, or other eukaryote (see, e.g., Bodnar et al., 1998, Science 279:349 and ~~copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178). Variants that lack at least one hTRT activity (e.g., telomerase catalytic activity) are used, inter alia, to inhibit telomerase activity in a cell (e.g., by acting as "dominant negative mutants"). The hTRT variants and polynucleotides encoding them, as described herein, are similarly useful in screening assays for identifying agents that modulate telomerase activity.

[0020] As used herein, an hTRT variant is considered to have a specified activity if the activity is exhibited by either the hTRT variant polypeptide without an associated hTR RNA or in an hTRT-hTR complex. Each of the hTRT activities described supra is also described in detail in ~~copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178.

[0027] As noted supra, the hTRT variant polypeptides of the invention comprising mutations (e.g., deletions) in the "non-essential" regions of the hTRT retain telomerase catalytic activity. These variants, and polynucleotides that encode them, are useful in any application for which other catalytically active hTRT proteins (e.g., wild-type hTRT proteins) or polynucleotides may be used, including, inter alia, in therapeutic, diagnostic, and screening uses. Exemplary uses of hTRT polypeptides and polynucleotides are described in additional detail in the afore cited ~~copending applications (e.g., U.S. application Ser. No. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178 ~~)~~.

[0028] In one embodiment, the hTRT variant of the invention is used to increase the proliferative capacity of a cell by, e.g., increasing telomerase activity in the cell (see, Bodnar et al. supra, and ~~copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178 for a detailed description of exemplary methods). Briefly, in one embodiment, a polynucleotide comprising (i) a sequence encoding the hTRT variant polypeptide; (ii) an operably linked promoter (e.g., a heterologous promoter); and, (iii) optionally polyadenylation and termination signals, enhancers, or other regulatory elements, is introduced into a target cell (e.g., by transfection, lipofection, electroporation, or any other suitable method) under conditions in which the hTRT variant polypeptide is expressed. The expression in the cell of the catalytically active hTRT variant of the invention results in increased proliferative capacity (e.g., an immortal phenotype).

[0029] In another embodiment, the hTRT variant is used for in vitro reconstitution (IVR) of a telomerase ribonucleoprotein (e.g., comprising the hTRT variant polypeptide and a template RNA, e.g., hTR) that has telomerase catalytic activity. In vitro reconstitution methods are described in, e.g., Weinrich et al., 1997, Nat Genet. 17:498, and ~~copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178. Briefly, in one embodiment, an expression vector encoding an hTRT variant of the invention is expressed in an in vitro expression system (e.g., a coupled transcription-translation reticulocyte lysate system such as that described in U.S. Pat. No. 5,324,637). In a particular embodiment, the hTRT variant polypeptide is coexpressed with hTR. In an alternative embodiment, the hTRT variant and hTR are separately expressed and then combined (mixed) in vitro. In the latter method, the hTR RNA and/or hTRT polypeptide may be purified before mixing. In this context, the hTRT polypeptide is "purified" when it is separated from at least one other component of the in vitro expression system, and it may be purified to homogeneity as determined by standard methods (e.g., SDS-PAGE). The in vitro reconstituted (IVR) telomerase has a variety of uses; in particular it is useful for identifying agents that modulate hTRT activity (e.g., drug screening assays).

[0032] The PCA$^-$ variant proteins and polynucleotides of the invention lacking telomerase catalytic activity are used in, inter alia, therapeutic, screening and other applications. For example, PCA$^-$ variants are useful as dominant negative mutants for inhibition of telomerase activity in a cell. In one embodiment, a PCA$^-$ variant is introduced into a cell (e.g., by transfection with a polynucleotide expression vector expressing the PCA$^-$ variant), resulting in sequestration of a cell component (e.g., hTR) required for accurate telomere elongation. Thus, for example, administration of a polypeptide that binds hTR, a DNA primer, a telomerase-associated protein, or other cell component, but which does not have telomerase catalytic activity, is used to reduce endogenous telomerase activity in the cell or to otherwise interfere with telomere extension (e.g., by displacing active telomerase from telomeric DNA). Similarly, in certain embodiments, a PCA$^-$ variant of the invention having one or several hTRT activities (i.e., other than processive telomerase catalytic activity) is used for screening for agents that specifically modulate (inhibit or activate) a telomerase activity other than telomerase catalytic activity. The use of hTRT variants as dominant negative mutants, and in other applications, is described in detail in ~~copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178.

[0036] Typically, the recombinant polynucleotide encoding an hTRT variant of the invention is linked to appropriate regulatory elements (e.g., promoters, enhancers, polyadenylation signals, and the like) and expressed in a cell free system (see, e.g., Weinrich et al., supra), in bacteria (e.g., E. coli), in ex vivo animal cell culture (see, e.g., Bodnar et al., supra), in animals or plants (e.g., transgenic organisms or in gene therapy applications), or by any other suitable method. Suitable expression systems are well known in the art and include those described in Weinrich et al., and Bodnar et al., both supra, and in ~~e.g., copending U.S. patent application Ser. Nos. 08/912,951 and 08/974,549~~ U.S. Patent Nos. 6,475,789 and 6,166,178.

[0051] ~~As used herein, a polypeptide region in a first polypeptide "corresponds" to a region in a second polypeptide when the amino acid sequences of the two regions, or flanking the two regions, are the same or substantially identical.~~ When comparing regions between a first and second polypeptide, ~~Sequences~~ sequences can be aligned by inspection (e.g., alignment of identical sequences) or by computer implemented alignment of the two sequences. Thus, for example, the residues 192 to 323 of the hTRT polypeptide having the sequence set forth in FIG. 1 "correspond" to residues in the same position in a hTRT polypeptide that differs from the FIG. 1 sequence due to polymorphic variation, or other mutations or deletions (e.g., when the two polypeptides are optimally aligned). Alignments may also be carried out using the GAP computer program, version 6.0 (Devereux et al, 1984, Nucl. Acid. Res. 12:387; available from the University of Wisconsin Genetics Computer Group, Madison, Wis.). The GAP program utilizes the alignment method of Needleham and Wunsch, 1970 J. Mol. Biol. 48: 443-453 as revised by Smith and Waterman, 1981, Adv. Appl. Math 2:482. The preferred default parameters for the GAP program include (1) the weighted comparison matrix of Gribskov and Burgess, 1986, Nucl. Acid. Res. 14:6745 as described by Schwartz and Dayhoff, eds., 1979, ATLAS OF PROTEIN SEQUENCE AND STRUCTURE, National Biomedical Research Foundation, pp. 353-358 (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Alternatively, alignments can be carried out using the BLAST algorithm, which is described in Altschul et al., 1990, J. Mol. Biol. 215:403-410 using as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, 1989, Proc. Natl. Acad. Sci. USA 89:10915); alignments (B) of 50, expectation (E) of 10, M=5, and N=-4. A modification of BLAST, the "Gapped BLAST" allows gaps to be introduced into the alignments that are returned (Altschul et al., 1997, Nucleic Acids Res 1:3389-3402). Software for performing BLAST analyses is publicly available through the internet website of the National Center for Biotechnology Information ~~(http://www.ncbi.nlm.nih.gov/)~~.

Please insert the following directly after paragraph [0051] of the application as published:

As used herein, "stringent hybridization conditions" or "stringency" refers to conditions in a range from about 5°C. to about 20°C. or 25°C. below the melting temperature (T_m) of the target sequence and a probe with exact or nearly exact complementarity to the target. As used herein, the melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands. Methods for calculating the T_m of nucleic acids are well known in the art (see, e.g., Berger and Kimmel (1987) METHODS IN ENZYMOLOGY, VOL. 152: GUIDE TO MOLECULAR CLONING TECHNIQUES, San Diego: Academic Press, Inc. and Sambrook et al. (1989) MOLECULAR CLONING: A LABORATORY MANUAL, 2ND ED., VOLS. 1-3, Cold Spring Harbor Laboratory hereinafter, "Sambrook", both incorporated herein by reference). As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41(\% G+C)$, when a nucleic acid is in aqueous solution at 1 M NaCl (see e.g., Anderson and Young, Quantitative Filter Hybridization in NUCLEIC ACID

HYBRIDIZATION (1985)). Other references include more sophisticated computations which take structural as well as sequence characteristics into account for the calculation of T_m. The melting temperature of a hybrid (and thus the conditions for stringent hybridization) is affected by various factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, and the like), and the concentration of salts and other components (e.g., the presence or absence of formamide, dextran sulfate, polyethylene glycol). The effects of these factors are well known and are discussed in standard references in the art, e.g., Sambrook, supra and Ausubel et al. supra. Typically, stringent hybridization conditions are salt concentrations less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion at pH 7.0 to 8.3, and temperatures at least about 30°C. for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C. for long probes (e.g., greater than 50 nucleotides). As noted, stringent conditions may also be achieved with the addition of destabilizing agents such as formamide, in which case lower temperatures may be employed.

As used herein, the term "substantial identity," "substantial sequence identity," or "substantial similarity" in the context of nucleic acids, refers to a measure of sequence similarity between two polynucleotides. Substantial sequence identity can be determined by hybridization under stringent conditions, by direct comparison, or other means. For example, two polynucleotides can be identified as having substantial sequence identity if they are capable of specifically hybridizing to each other under stringent hybridization conditions. Other degrees of sequence identity (e.g., less than "substantial") can be characterized by hybridization under different conditions of stringency. Alternatively, substantial sequence identity can be described as a percentage identity between two nucleotide (or polypeptide) sequences. Two sequences are considered substantially identical when they are at least about 60% identical, preferably at least about 70% identical, or at least about 80% identical, or at least about 90% identical, or at least about 95% or 98% to 100% identical. Percentage sequence (nucleotide or amino acid) identity is typically calculated by determining the optimal alignment between two sequences and comparing the two sequences. For example an exogenous transcript used for protein expression can be described as having a certain percentage of identity or similarity compared to a reference sequence (e.g., the corresponding endogenous sequence). Optimal alignment of sequences may be conducted using the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48: 443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection. The best alignment (i.e., resulting in the highest percentage of identity) generated by the various methods is selected. Typically these algorithms compare the two sequences over a "comparison window" (usually at least 18 nucleotides in length) to identify and compare local regions of sequence similarity, thus allowing for small additions or deletions (i.e., gaps). Additions and deletions are typically 20 percent or less of the length of the sequence relative to the reference sequence, which does not comprise additions or deletions. It is sometimes desirable to describe sequence identity between two sequences in reference to a particular length or region (e.g., two sequences

may be described as having at least 95% identity over a length of at least 500 basepairs). Usually the length will be at least about 50, 100, 200, 300, 400, or 500 basepairs, amino acids, or other residues. The percentage of sequence identity is calculated by comparing two optimally aligned sequences over the region of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, or U) occurs in both sequences to yield the number of matched positions, and determining the number (or percentage) of matched positions as compared to the total number of bases in the reference sequence or region of comparison.

Please make the following change to the ABSTRACT:

The invention provides compositions and methods related to human telomerase reverse transcriptase (hTRT), the catalytic protein subunit of human telomerase. Catalytically active and inactive human telomerase reverse transcriptase variants comprising deletions or other mutations are provided.